EMMA ID: *13430*
Gene: *Phactr4*
Common name: *Phactr4-em1_2*
Allele: *Phactr4* $^{em1(IMPC)Hmgu}$

Allele Information

For more information on production,guides and mutation, search for gene/project, go to project summary, go to production plan, go to production outcome and "more details"
https://www.gentar.org

IMPC mouse phenotype data, search by the gene name
http://www.mousephenotype.org/

Genotyping Information

Genotyping by end-point PCR based on gel is composed of a genespecific short range PCR using primers on wild type allele and a mutant allele-specific short range PCR. The combined results show the genotype of the mice. For example: mutant positive, wild type positive = Heterozygous. In addition to the expected product, the mutant assay may also amplify the endogenous wild type sequence, which will appear as a larger band on an agarose gel. The presence of this extra band will depend on the size of the original deletion.

Phactr4-em1_2 contains a -10bp deletion and therefore needs to be sequenced.

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wild type	Phactr4 for2	Phactr4 rev 2	339
Mutant	same as wt	same as wt	329

Primer sequences

Primer Name	Sequence 5' --> 3'
Phactr4 for2	tggcagcagtgttagcactggcaggag
Phactr4 rev 2	tactccacccccaagcagggcccttg

PCR setup (LongAmp® Taq DNA Polymerase)

Component	Volume (µl) 1x
DNA (~ 50-100 ng)	2-4
100% DMSO	0,4
PCR-Buffer (5x)	4
DNTP mix (10 mM)	0,5
Primer 1 (10 pmol/µl)	1
Primer 2 (10 pmol/µl)	1
Primer 3 (10 pmol/µl)	1
Taq Polymerase (2,5U/µl	0,5
H2O*	7,6
Final volume	20

*** The amount of H2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	94°C	3 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 68-58 (↓1°C/Cycle) 65°C	30 sec 20 sec 1 min	39
3 Polymerisation	65°C	10 min	1
4 Cooling	4°C	hold	1

Touch-Down cycling protocol: first 10 cycles anneal at 68°C, decreasing 1°C per cycle, next 30 cycles aneal at 58°C.
These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.

Sequence / mutation

Wt:

ccggaacctcctttgcttcacaagaagaggacaagggtctcttgggggcagcagaggctgcttaggtgcatgctcagga

Mut:

ccggaacctcctttgcttcacaagaagaggacaagggggcagcagaggctgcttaggtgcatgctcagga